Chromosomal abnormalities in infertile men and preimplantation embryos

Dul, Elsbeth

CHAPTER 7

General discussion and recommendations

This thesis focuses on chromosomal abnormalities in infertile men and in preimplantation embryos.

After the general introduction in chapter 1, the next three chapters discuss the guidelines on screening of infertile men for chromosomal abnormalities. In chapter 2 we have demonstrated that the risk of chromosomal abnormalities is not correlated to sperm concentration, although the risk is significantly higher in azoospermic men, compared to non-azoospermic infertile men. In chapter 3 we present a model that can be used to identify men at high risk for chromosomal abnormalities, helping in reducing the number of infertile men that are karyotyped. In chapter 4 we calculate the numbers needed to screen to prevent adverse pregnancy outcomes. Based on the studies in these three chapters we recommend restricting karyotyping in infertile couples to azoospermic men only.

In the studies on chromosomal abnormalities in preimplantation embryos, presented in chapters 5 and 6, our aim was to study factors that might contribute to improving the outcome of PGD. In chapter 5 we studied whether culturing embryos in a reduced oxygen environment could result in lower chromosomal mosaicism rates. We did not find a significant effect of oxygen tension on sex chromosomal mosaicism. In chapter 6 we studied a cohort of PGD couples with a reciprocal translocation, in search of cytogenetic factors predicting the percentage of unbalanced embryos. With a higher ratio of the relative sizes of the translocated segments (more asymmetry), less balanced embryos are produced.

Genetic screening policy in infertile men

Number needed to screen

Contrary to previous publications, we have shown that in men eligible for ICSI, the prevalence of chromosomal abnormalities does not increase with declining sperm counts. Azoospermic men, however, carry significantly more often a chromosomal abnormality compared to non-azoospermic infertile men (15.2% *vs.* 2.3%; OR 7.7). This is also reflected in the difference in NNS between azoospermic and non-azoospermic men. The number of azoospermic men that needs to be screened to prevent one miscarriage (80-88) or one child with congenital anomalies (790-3951) was considerably lower compared with the NNS in the non-azoospermic group (315-347 and 2543-12723, respectively). The decision which screening policy is most optimal not only depends on the NNS, but a number of factors should be considered, such as financial aspects, psychological considerations and the perspective of the patient.

Financial considerations

The Dutch guideline on screening infertile men for chromosomal abnormalities was very strict at the introduction of ICSI, by making karyotyping a prerequisite before starting treatment (NVOG, 1999). The main reason for this was to avoid increasing the number of miscarriages and children with congenital anomalies being born. The lifetime costs of the latter impose a great burden on society. However, screening for chromosomal abnormalities in infertile men is also costly, especially with low prevalences. The number of infertile men presenting to fertility clinics can be estimated from the number of ICSI cycles performed in the Netherlands per year at about 3900 (NVOG, 2014). Several of these men will be azoospermic and underwent ICSI-PESA or ICSI-TESE. The estimated number of PESA and TESE procedures is 900/year. The costs per karyotype analysis are approximately €800. If non-azoospermic infertile men are no longer routinely karyotyped, the reduction of costs in the Netherlands per year will be approximately 3000 x €800 = €2,400,000. When applying this strategy, chromosomal abnormalities will not be detected in non-azoospermic infertile men. In this group of infertile men the prevalence of chromosomal abnormalities with increased risk for a child with congenital anomalies or miscarriage is only 1.0% (Dul *et al.*, 2012). In 3000 men, this means that annually one or two children with congenital anomalies will be born due to not karyotyping the infertile non-azoospermic father.

A study in the USA developed a nomogram to predict the chance of positive testing for chromosomal abnormalities and AZF deletions in 278 non-obstructive azoospermic and severely oligozoospermic men (concentration < 2.5 million/ml) (Khurana *et al.*, 2014). The model included sperm concentration and motility, testicular volume and testosterone level. The cost of karyotyping and AZF deletion testing were $2070 and $517, respectively. The authors tested different cut-off levels in the model. With the most cost-effective cut-off, a cost saving of 45% would be realized. This would be at the expense of missing 15.4% of the genetic abnormalities in the population. Since the screened population consisted of azoospermic and severely oligozoospermic men, the genetic aberrations that would be missed will mostly be AZF deletions and gonosomal abnormalities without major consequences for the offspring. Not detecting these aberrations because of stricter screening criteria will have little impact on the number of children with congenital anomalies. What this study illustrates, is that, even in the very high risk population of non-obstructive azoospermic men, attempts are made to reduce the costs of extensive screening. More research is needed to gain insight into the costs and consequences of the missed diagnoses, because it is not easily calculated how many extra children with congenital anomalies will be born if restrictions are made to karyotyping infertile men. A study on the outcome of spontaneous pregnancies in carriers of a structural autosomal abnormality and non-carriers with recurrent miscarriages revealed a comparable risk of live born children with congenital anomalies, although the risk of miscarriage was higher in the carrier group (Franssen *et al.*, 2006). Furthermore, the possibility of prenatal screening and diagnosis is available in most

countries. These methods can detect many congenital anomalies, even in cases where the paternal karyotype is not known, and can therefore (partly) replace the screening of infertile men for chromosomal abnormalities. When calculating the costs of children born with an unbalanced karyotype due to an altered screening strategy in infertile men, one should correct for the chance that these children are prenatally detected by the current screening programs, especially the structural ultrasound screening. While doing so, two factors should be taken into account. Firstly, differences in the uptake of prenatal screening between populations have been described. For example, people living in more rural areas seem less inclined to opt for screening for Down syndrome than people living in urban areas (Bakker *et al.*, 2012). Secondly, prenatal screening and diagnosis do not exclude the birth of a child with congenital anomalies, as some structural anomalies are too small to be recognized on ultrasound, or not related to the chromosomal abnormalities that are screened for. Moreover, the costs of a child born with congenital anomalies due to not karyotyping the father are difficult to assess, because of the broad spectrum of congenital anomalies that may occur. Nonetheless, in modeling screening strategies, the costs of children with congenital anomalies and the costs of the alternative strategies to detect them prenatally need to be taken into consideration.

Psychological considerations and the perspective of the patient

Apart from identifying the risk of anomalies in the offspring, karyotyping may also be part of the diagnostic work-up in infertile men, to differentiate between idiopathic infertility and a known genetic cause of infertility. Furthermore, knowing the cause of infertility can help in the coping process of the couple, and thus it is important to take into consideration the wishes of the couple regarding genetic tests and what significance the results have to them. Whether the knowledge of a chromosomal abnormality affects the reproductive choice of a couple has been the topic of several studies. Previous studies have shown that after extensive counseling, 82% of couples with chromosomal abnormalities proceeded with ART (Giltay *et al.*, 1999; Riccaboni *et al.*, 2008; Stegen *et al.*, 2012; Tiboni *et al.*, 2011). In our own cohort of men with chromosomal abnormalities, most non-azoospermic men underwent ICSI as well. This indicates that they accept the increased risk of miscarriage or viable unbalanced offspring. In case of an increased risk of children with congenital anomalies, prenatal diagnosis was available. However, in the combined studies in the literature, only 44% of the couples with an ongoing pregnancy (N=25) underwent invasive prenatal diagnosis. These revealed only balanced or normal karyotypes, and no children with congenital anomalies were born (Giltay *et al.*, 1999; Riccaboni *et al.*, 2008; Stegen *et al.*, 2012; Tiboni *et al.*, 2011). The low uptake of invasive prenatal diagnosis in a pregnancy acquired by ART is probably caused by the risk of miscarriage of the procedure. It is quite possible that couples that refrain from invasive prenatal diagnostics, rely on non-invasive procedures for the screening for congenital anomalies. We have no information on the extent of (first trimester) serum screening or advanced ultrasound during the pregnancies in the studies mentioned.

Non-invasive prenatal screening (NIPT) is rapidly replacing invasive prenatal diagnostics. NIPT is a genome-wide fetal aneuploidy detection by DNA sequencing of maternal plasma, without a risk of miscarriage due to the procedure (Bianchi *et al.*, 2012). This will increase the number of carriers of chromosomal abnormalities choosing prenatal testing.

AZF deletions

In addition to the recommendations guidelines provide on screening infertile men for chromosomal abnormalities, they advise to screen for AZF deletions. In general, screening for AZF deletions is advised in severe oligozoospermic and azoospermic men, mainly to find the cause of infertility. A man that carries an AZF deletion does not have a higher risk of a child with congenital anomalies or a miscarriage. In a study among 28 carriers of an AZF deletion, approximately 80% of the couples underwent ICSI, despite the fact that they were counseled that all male offspring will have the AZF deletion and most likely the concomitant infertility (Nap *et al.*, 1999). In another study, nearly all men with an AZF deletion proceeded to ICSI treatment, provided sperm was available (Patrat *et al.*, 2010).

With the introduction of ICSI with surgically retrieved sperm, the detection of an AZF deletion can be useful in determining the prognosis of a TESE procedure in azoospermia. When there are complete AZFa and AZFb microdeletions, the likelihood of sperm retrieval is almost zero, while in men with an isolated AZFc deletion the likelihood is approximately 50% (Jungwirth *et al.*, 2013).

In our study cohort 1156 men eligible for ICSI or with azoospermia were tested for AZF deletions. We found a prevalence of 1.0%. Eight non-azoospermic men had an AZFc deletion, and they all proceeded with ICSI. Of the three azoospermic men with an AZF deletion, one had an AZFb deletion, one an AZFb deletion and Klinefelter syndrome and one man had a complex Y chromosome anomaly with AZFb partly and AZFc completely missing. These three men were not eligible for TESE.

Thus, in general, non-azoospermic infertile men with an AZF deletion tend to proceed with ICSI and the adverse effect on their offspring seems to be limited to infertility in their sons. Therefore, screening for AZF deletions should not be standard in the diagnostic work-up, but restricted to azoospermic men if TESE is considered, to exclude AZFa and AZFb deletions.

Discrepancies in existing guidelines

Since the start of the studies described in this thesis, nearly all societies of reproductive medicine have issued an update of their guideline on male infertility. However, in the past five to ten years, not much has changed in these guidelines regarding the recommendations on genetic screening of infertile men. The NICE guideline has not changed any of their recommendations (NICE, 2013). In 2010, a new Dutch guideline was issued, stating that chromosomal and Y chromosome analysis can be considered in

men with non-obstructive azoospermia or extreme oligoasthenoteratozoospermia (TMSC < 1 million). In this new guideline, an indication for ICSI treatment alone is no longer a reason for karyotyping. Most importantly, the decision on genetic testing is made by the couple and the clinician together, based on counseling (NVOG, 2010).

Gynaecologists and clinical geneticists working in reproductive medicine in the Netherlands have adopted a more restrictive screening policy, to which our studies have contributed. Karyotyping in infertile men is now restricted to azoospermic men, as the numbers needed to screen, and therefore the costs, in non-azoospermic infertile men are relatively high. This advice has resulted in a significant cost reduction. However, andrologists, urologists or gynaecologists and clinical geneticists in other countries may adhere to different guidelines. The European Association of Urology has altered the indication for both karyotyping and screening for AZF deletions in their new guideline of 2013: "Karyotyping should be offered to all men with damaged spermatogenesis (spermatozoa < 10 million/mL) who are seeking fertility treatment by IVF. For men with severely damaged spermatogenesis (spermatozoa < 5 million/mL), testing for AZF deletions is strongly advised". However, the guideline also states that, as these men and their male children are unlikely to have any phenotypic abnormality other than impaired spermatogenesis, it is reasonable to take into account the costs and limitations of current testing methods and to discuss this with the couple (Jungwirth *et al.*, 2013).

Based on these considerations we recommend to restrict karyotyping and DNA analysis for AZF deletions to azoospermic men, when they apply for ICSI with surgically retrieved sperm. Only in case of recurrent miscarriage, or a child with congenital anomalies in their family, karyotyping should be considered in non-azoospermic infertile men as well.

A further reduction of performing genetic tests may be established when future studies address the difference in prevalence of chromosomal abnormalities between men with obstructive and non-obstructive azoospermia. We already found that in hypergonadotropic azoospermic men the prevalence of chromosomal abnormalities is significantly higher (23.1%) compared to normogonadotrophic azoospermic men (6.7%). Since ART in azoospermic men becomes more and more available, it is important to identify the subpopulation with a high risk of chromosomal abnormalities.

Preimplantation genetic diagnosis

The ultimate goal of PGD is to increase the chance of a healthy child in couples that carry a genetic abnormality. PGD is performed to prevent a pregnancy with a fetus affected by that abnormality, or to prevent recurrent miscarriages. Although most PGD couples are fertile, pregnancy rates are comparable to those in infertile couples in regular IVF programs. In the second part of this thesis we have addressed two factors that may influence the outcome of PGD: chromosomal mosaicism in the embryo and high numbers of unbalanced embryos in translocation carriers.

Mosaicism

In a pilot study on the influence of oxygen tension on rates of sex chromosomal mosaicism in human embryos, we did not find a significant difference in mosaicism rate between both study groups. These findings contrast with those of a study in mice by Bean *et al.* (2002), who found a lower mosaicism rate in embryos cultured in a reduced oxygen environment.

In general, aneuploidy, full or mosaic, is not common in mouse embryos, contrary to human embryos. In fact, the cleavage stage of human embryonic development stands out because of the high rate of chromosomal anomalies, including mosaicism. Accumulated data show that on average 60% of human embryos created by IVF have at least one aneuploid cell by the time they reach day 3 (summarized by Mantzouratou and Delhanty, 2011). These high rates may partly explain the relatively poor fecundity rate of humans as a species and the low success rates of ART. In PGD, mosaicism can seriously hamper the accuracy of the method and thereby the pregnancy rates. The conditions in which embryos are cultured may influence the rate of mosaicism, but the optimal conditions that give the lowest rate of mosaicism, and thereby possibly the highest success rates in IVF and PGD, are not known.

The study by Bean *et al.* (2002) in mice showed an influence of oxygen tension on mosaicism, but we could not find a significant difference in human embryos in our study (Chapter 5). The influence of culture conditions (*e.g.* culture medium, oxygen tension) might be aggravated when the embryo has undergone a blastomere biopsy in accordance with the PGD procedure. Most studies on chromosomal mosaicism are performed in spare embryos. The use of spare, or supernumerary embryos by definition means using embryos that are inferior in some respect. They can be spare because of poor morphology or development, so preference has been given to better developed embryos for transfer or cryopreservation. In PGD they can also be spare because they are affected with the genetic disorder for which PGD is performed. These factors may all individually influence the mosaicism or aneuploidy rate, which hinders studies in mosaicism in human embryos.

The developmental stage of the embryo may be of influence on mosaicism rate. It has been stated that in blastocysts the rate of mosaicism is lower than in cleavage stage embryos. Theoretically, the development of cells with abnormalities is hampered, which means that by natural selection less abnormal cells reach the blastocyst stage. This is called the possibility of self-correction of aneuploidy within the embryo (Fiorentino *et al.*, 2011; Fragouli and Wells, 2011; Fragouli *et al.*, 2011). Furthermore, although mosaicism is also present at the blastocyst stage, compared to the cleavage stage embryo more cells of the blastocyst can be analyzed, allowing selection against fully aneuploid embryos or embryos with such a high count of aneuploid cells that it severely decreases the embryo's survival chance (Fragouli and Wells, 2011). Despite these two favourable factors of blastocysts, the level of mosaicism at which an embryo is still

viable is unknown, as well as its property to develop into an affected fetus. This means that, even in PGD at the blastocyst stage, mosaicism can cause discarding of eventually euploid embryos or transfer of aneuploid embryos. In blastocysts the trophectoderm is biopsied, as studies suggest that trophectoderm samples provide an accurate indication of the chromosome constitution of the inner cell mass in the vast majority of cases (summarized by Fragouli *et al.*, 2011). However, from prenatal diagnosis it is known that placental mosaicism exists, and the trophectoderm could have a different level of mosaicism than the inner cell mass. In PGD, this could lead to inappropriately selecting an embryo for transfer or discarding it.

It has been stated that FISH technology may overestimate the rate of mosaicism in cleavage stage embryos (Treff *et al.*, 2010). In FISH analysis, the estimated probe accuracy is 92-99% in control cells. A technical limitation of the FISH procedure is that scoring errors may occur due to loss or damage of nuclear material, (partial) overlapping of signals, presence of split or diffuse signals, hybridization failure or probe inefficiency. These technical limitations may cause the designation of a blastomere as aneuploid while in fact it is not, and *vice versa*. Despite these possible scoring errors, FISH analysis of interphase nuclei has a positive predictive value of 83% (DeUgarte *et al.*, 2008; Ruangvutilert *et al.*, 2000; Scriven and Bossuyt, 2010; Wilton *et al.*, 2009). Novel techniques that are less sensitive to interpretation errors, such as those based on array technology, may have higher accuracy rates than FISH. In this respect, several studies have been done using array comparative genomic hybridization (array-CGH; aCGH) in 24-chromosome aneuploidy screening (PGS) in blastocysts (Fiorentino *et al.*, 2014; Greco *et al.*, 2014; Rubio *et al.*, 2013; Yang *et al.*, 2014). Currently, array-CGH is used in PGD in cleavage stage embryos, and it is considered to be less error-prone (Fiorentino *et al.*, 2011; Harper and Sengupta, 2012; Keltz *et al.*, 2013). It is to be expected that this technique will completely replace FISH in the near future, although limited data is available on the diagnostic accuracy of this test (Mastenbroek and Repping, 2014). Nevertheless, even array methods show mosaicism to be present at high levels (32.4 - 84%) in spare human preimplantation embryos, with the highest levels present in cleavage stage embryos (Fiorentino *et al.*, 2011; Fragouli *et al.*, 2011; Mertzanidou *et al.*, 2013; Wells and Delhanty, 2000).

All 24 chromosomes are tested in the array technique, which means other chromosomal aneuploidies can be detected than just the abnormality for which PGD is offered. It remains to be decided what to do with this additional information, and which embryo should be transferred: embryos normal for the abnormality for which PGD is offered, embryos without any (chromosomal) abnormality, or only to transfer those that are normal for the abnormality for which PGD is offered and diploid for chromosomes 21, 18 and 13. Should the test results of all chromosomes be known, to the doctor or the couple, if one is not interested in any additional data? Another question is what abnormalities are accepted to be transferred, if no completely normal embryos are available. As the level of mosaicism increases with the number of chromosomes tested (van Echten-Arends *et al.*, 2011), it is to be expected that more embryos will be classified as abnormal, also resulting in less transferable embryos and possibly causing a decrease in pregnancy rates. The implementation of array and only transferring completely normal embryos is to be preferred if this reduces implantation failure and miscarriage rates and increases live birth rates, but this remains to be proven (Mastenbroek and Repping, 2014). Furthermore, with a full array of all 24 chromosomes, the sex of the embryo will always be known, which brings into discussion the ethical issues surrounding social sexing (Hens *et al.*, 2013).

In conclusion, chromosomal mosaicism has a high prevalence in human preimplantation embryos. This may influence the accuracy of PGD. Mosaicism may be influenced by culture conditions or stage of embryo development, but optimal conditions remain to be determined. Novel techniques have not yet proven to improve the outcome of PGD, and raise dilemmas as to which embryos to transfer.

Translocations

Some translocations are associated with infertility and/or high miscarriage rates; for others, there may be a risk of offspring with mental and physical disability due to unbalanced segregation of the translocation chromosomes at meiosis resulting in sperm or oocytes with a chromosome imbalance. Prenatal diagnosis can enable early diagnosis with the option of termination of the pregnancy in case of an affected fetus. PGD is used for translocation carriers to minimize the risk of having an affected child or the distress of pregnancy termination, and to reduce the risk of miscarriage due to abnormal segregation of the translocation chromosomes.

From the ESHRE PGD data collections it can be gathered that, compared to the other PGD indications, PGD in reciprocal translocation carriers results in the lowest pregnancy rates per oocyte retrieval (14.5% *vs.* 22% in PGD for single gene disorders) (Goossens *et al.*, 2012). This is due to the fact that reciprocal translocation carriers produce a large number of abnormal gametes, due to unfavourable segregation of the chromosome pairs involved in the translocation from the quadrivalent they form during meiosis. Only 2 of the possible 32 segregation products result in a normal or balanced genotype. The large number of abnormal gametes results in a high number of unbalanced embryos that are not suitable for transfer. This has led to a debate on whether or not to offer PGD to reciprocal translocation carriers. Several studies have argued that the risk of unbalanced viable offspring is low (0.4-1.1%), if the ascertainment of the carrier status was through recurrent miscarriage (Carp *et al.*, 2004; Franssen *et al.*, 2011; Franssen *et al.*, 2006). Although the risk of another miscarriage is higher if the subsequent pregnancy is spontaneously conceived, compared to conception by PGD, the long-term chance of a live birth is equal (33-60% and 0-100%, respectively) (reviewed by Franssen *et al.*, 2011). There is much discussion on how to compare the time to a successful pregnancy with PGD or spontaneous conception in these studies. With spontaneous pregnancies time is

measured in months, with PGD in cycles. However, the technical work-up and waiting-lists in PGD can delay the actual procedure with many months. The time needed for the technical work-up can be shortened using array analysis instead of FISH. An advantage of array over FISH for translocations is that it does not require preclinical validation before each cycle (Chang *et al.*, 2011). Another delay in achieving a successful pregnancy by PGD may be due to the production of predominantly unbalanced embryos, which leads to the need of many cycles to have an embryo transferred. Array provides information on aneuploidies not related to the translocation as well, and may result in even less embryos suitable for transfer. On the other hand, the distress of experiencing multiple miscarriages can be a reason for a couple to undergo the invasive and time-consuming procedure of PGD. Studies in translocation carriers have shown that pregnancy loss can be significantly reduced to 13% with PGD, while with natural conception it was 88.5% (Fischer *et al.*, 2010; Otani *et al.*, 2006). For translocation carriers with a high risk of viable unbalanced offspring, or if assisted conception is already needed because of infertility, PGD is a reasonable option to prevent children with congenital anomalies and further miscarriages (Scriven *et al.*, 2013). For couples without fertility issues, the costs and benefits of IVF-PGD should be considered, *e.g.* the invasive and costly IVF-procedure, the limited change in live birth rate, the possible reduction in time to a normal or balanced pregnancy and in miscarriage rate.

There may be a subgroup of reciprocal translocation carriers that has a higher chance of producing balanced embryos, and therefore a higher success rate in PGD. If this subgroup can be identified before PGD treatment is offered, couples can decide whether or not to undergo PGD based on this counseling. In the counseling predictors such as maternal age, ovarian reserve, (in)fertility of the couple, gender of the translocation carrier, and cytogenetic characteristics of the translocation are used to estimate the success of PGD (Feenstra *et al.*, 2006; Schinzel, 2001; Stengel-Rutkowski *et al.*, 1988). We hypothesized that with increasing imbalance between the two translocation chromosomes, the percentage of balanced embryos would decline. In our study into cytogenetic characteristics of translocations, no predictors were identified other than the ratio of the relative sizes of the translocated segments. This suggests that more unbalanced embryos are produced when the quadrivalent is more asymmetrical. A similar study in a recent publication confirms the results of our study (Zhang *et al.*, 2014). The fact that no other predictors were identified may have been partly caused by the relatively small number of couples with a translocation in our cohort. Further studies are therefore needed in larger cohorts, such as the ESHRE PGD data collection.

The changing genetic landscape

In the studies of this thesis the current genetic techniques were used. However, a rapid transition is taking place in clinical genetics from karyotyping to array and even whole exome and whole genome sequencing. In whole exome sequencing (WES) all gene-coding regions – an estimated 1% of all human DNA- are screened for variants. This technology has largely been used for research into the basis of genetic disease and in clinical setting has shown benefit to individuals with rare diseases, where specific genetic tests were unable to determine the etiology of the disease. WES is more and more replacing standard genetic tests, because of better availability and lower costs. This has the potential to reveal a large amount of additional data, such as carrier status of recessive disorders, genetic risk factors for cancers or other acquired diseases. Although WES, like array, can detect AZF deletions, it cannot identify balanced translocations. This means that if WES is applied in the diagnostics of male infertility, karyotyping is still needed.

Whole genome sequencing (WGS) enables the determination of a person's complete DNA sequence. The advantage WGS has over WES is that it can detect balanced reciprocal translocations. At the moment it cannot detect Robertsonian translocations. Of the translocations we detected in our studies, 36% were Robertsonian translocations. Moreover, WGS is still an expensive technique (€5,000 – 10,000) and it generates large amounts of data, for which the interpretation is complex.

The major advantage of WES and WGS is that many genes can be investigated at the same time. However, up until this moment only a few monogenic causes of male infertility have been diagnosed by whole genome approaches (Aston, 2014). Although this may rapidly change in the near future, at the moment there does not seem to be a place for WES or WGS in the routine clinical work-up of infertile men.

It is quite possible that WES will be incorporated in the newborn screening for childhood diseases some time in the future. There is talk of centrally storing neonatally obtained exome or genome data in order to consult them for specific clinical questions whenever needed. In the newborn period these data can be used for serious but treatable disorders, later in life for diagnostics and personalized medicine (pharmacogenetics), or even for preconception advice (carrier status of recessive disorders). Thus, physicians may use the genomic information to minimize the impact of a disease or to avoid it completely by using preventive medicine or reproductive options.

However, this scenario of broad application of WES or WGS in the general population will take at least a decade to become reality, due to still unresolved technical and ethical dilemmas. Until that day, there will be a need for selective screening strategies as described in this thesis in daily clinical practice.

Recommendations

- Restrict screening for chromosomal abnormalities and AZF deletions to men with azoospermia.
- Karyotype non-azoospermic infertile men only in case of a history of recurrent miscarriage or a family history of a child with congenital anomalies.
- Identify predictive factors of chromosomal abnormalities in the subgroup of azoospermic men. A further reduction in karyotype analyses might be made by selecting those azoospermic men with the highest risk of chromosomal abnormalities.
- Maintain thorough counseling of carriers of a reciprocal translocation on reproductive options before PGD is offered.
- Confirm the association between asymmetrical reciprocal translocations and high percentage of unbalanced embryos in the large cohort of the ESHRE PGD data collection. If this further research confirms the predictive value of the ratio of the relative sizes of the translocated segments, it can be used in the counseling of couples that carry a reciprocal translocation before PGD treatment is considered.

References

Aston KI. Genetic susceptibility to male infertility: news from genome-wide association studies. *Andrology* 2014;**2**:315-321.

Bakker M, Birnie E, Pajkrt E, Bilardo CM and Snijders RJ. Low uptake of the combined test in The Netherlands--which factors contribute? *Prenat Diagn* 2012;**32**:1305-1312.

Bean C, Hassold T, Judis L and Hunt P. Fertilization in vitro increases non-disjunction during early cleavage divisions in a mouse model system. *Hum Reprod* 2002;**17**:2362-2367.

Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP and MatErnal BLood IS Source to Accurately diagnose fetal aneuploidy (MELISSA) Study Group. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol* 2012;**119**:890-901.

Carp H, Feldman B, Oelsner G and Schiff E. Parental karyotype and subsequent live births in recurrent miscarriage. *Fertil Steril* 2004;**81**:1296-1301.

Chang LJ, Chen SU, Tsai YY, Hung CC, Fang MY, Su YN and Yang YS. An update of preimplantation genetic diagnosis in gene diseases, chromosomal translocation, and aneuploidy screening. *Clin Exp Reprod Med* 2011;**38**:126-134.

DeUgarte C, Li M, Surrey M, Danzer H, Hill D and DeCherney A. Accuracy of FISH analysis in predicting chromosomal status in patients undergoing preimplantation genetic diagnosis. *Fertil Steril* 2008;**90**:1049-1054.

Dul EC, van Echten-Arends J, Groen H, Dijkhuizen T, Land JA and van Ravenswaaij-Arts CM. Chromosomal abnormalities in azoospermic and non-azoospermic infertile men: numbers needed to be screened to prevent adverse pregnancy outcomes. *Hum Reprod* 2012;**27**:2850-2856.

Feenstra I, Fang J, Koolen DA, Siezen A, Evans C, Winter RM, Lees MM, Riegel M, de Vries BBA, van Ravenswaaij CMA *et al*. European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations (ECARUCA) an online database for rare chromosome abnormalities. *Eur J Med Genet* 2006;**49**:279-291.

Fiorentino F, Bono S, Biricik A, Nuccitelli A, Cotroneo E, Cottone G, Kokocinski F, Michel CE, Minasi MG and Greco E. Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. *Hum Reprod* 2014;**29**:2802-2813.

Fiorentino F, Spizzichino L, Bono S, Biricik A, Kokkali G, Rienzi L, Ubaldi FM, Iammarrone E, Gordon A and Pantos K. PGD for reciprocal and Robertsonian translocations using array comparative genomic hybridization. *Hum Reprod* 2011;**26**:1925-1935.

Fischer J, Colls P, Escudero T and Munné S. Preimplantation genetic diagnosis (PGD) improves pregnancy outcome for translocation carriers with a history of recurrent losses. *Fertil Steril* 2010;**94**:283-289.

Fragouli E and Wells D. Aneuploidy in the human blastocyst. *Cytogenet Genome Res* 2011;**133**:149-159.

Fragouli E, Alfarawati S, Daphnis D, Goodall N, Mania A, Griffiths T, Gordon A and Wells D. Cytogenetic analysis of human blastocysts with the use of FISH, CGH and aCGH: scientific data and technical evaluation. *Hum Reprod* 2011;**26**:480-490.

Franssen MTM, Musters AM, van der Veen F, Repping S, Leschot NJ, Bossuyt PMM, Goddijn M and Korevaar JC. Reproductive outcome after PGD in couples with recurrent miscarriage carrying a structural chromosome abnormality: a systematic review. *Hum Reprod Update* 2011;**17**:467-475.

Franssen MT, Korevaar JC, van der Veen F, Leschot NJ, Bossuyt PM and Goddijn M. Reproductive outcome after chromosome analysis in couples with two or more miscarriages: index [corrected]-control study. *BMJ* 2006;**332**:759-763.

Giltay JC, Kastrop PM, Tuerlings JH, Kremer JA, Tiemessen CH, Gerssen-Schoorl KB, van der Veen F, de Vries J, Hordijk R, Hamers GJ *et al*. Subfertile men with constitutive chromosome abnormalities do not necessarily refrain from intracytoplasmic sperm injection treatment: a follow-up study on 75 Dutch patients. *Hum Reprod* 1999;**14**:318-320.

Goossens V, Traeger Synodinos J, Coonen E, De Rycke M, Moutou C, Pehlivan T, Derks-Smeets IAP and Harton G. ESHRE PGD Consortium data collection XI: cycles from January to December 2008 with pregnancy follow-up to October 2009. *Hum Reprod* 2012;**27**:1887-1911.

Greco E, Bono S, Ruberti A, Lobascio AM, Greco P, Biricik A, Spizzichino L, Greco A, Tesarik J, Minasi MG *et al*. Comparative genomic hybridization selection of blastocysts for repeated implantation failure treatment: a pilot study. *Biomed Res Int* 2014;**2014**:457913.

Harper JC and Sengupta SB. Preimplantation genetic diagnosis: state of the art 2011. *Hum Genet* 2012;**131**:175-186.

Hens K, Dondorp W, Handyside AH, Harper J, Newson AJ, Pennings G, Rehmann-Sutter C and de Wert G. Dynamics and ethics of comprehensive preimplantation genetic testing: a review of the challenges. *Hum Reprod Update* 2013;**19**:366-375.

Jungwirth A, Diemer T, Dohle GR, Giwercman A, Kopa Z, Tournaye H and Krausz C. *European Association of Urology: Guidelines on Male Infertility*, 2013. Available online: http://www.uroweb.org/gls/pdf/16_Male_Infertility_LR.pdf

Keltz MD, Vega M, Sirota I, Lederman M, Moshier EL, Gonzales E and Stein D. Preimplantation genetic screening (PGS) with Comparative genomic hybridization (CGH) following day 3 single cell blastomere biopsy markedly improves IVF outcomes while lowering multiple pregnancies and miscarriages. *J Assist Reprod Genet* 2013;**30**:1333-1339.

Khurana KK, Baker K, Gao T and Sabanegh ES,Jr. The economic burden of genetic tests for the infertile male: a pilot algorithm to improve test predictive value. *J Urol* 2014;**191**:1066-1071.

Mantzouratou A and Delhanty JDA. Aneuploidy in the human cleavage stage embryo. *Cytogenet Genome Res* 2011;**133**:141-148.

Mastenbroek S and Repping S. Preimplantation genetic screening: back to the future. *Hum Reprod* 2014;**29**:1846-1850.

Mertzanidou A, Spits C, Nguyen HT, Van de Velde H and Sermon K. Evolution of aneuploidy up to Day 4 of human preimplantation development. *Hum Reprod* 2013;**28**:1716-1724.

Nap AW, Van Golde RJ, Tuerlings JH, De Sutter P, Pieters MH, Giltay JC, Kastrop PM, Braat DD and Kremer JA. Reproductive decisions of men with microdeletions of the Y chromosome: the role of genetic counselling. *Hum Reprod* 1999;**14**:2166-2169.

National Institute for Health and Clinical Excellence. *Fertility: Assessment and treatment for people with fertility problems*, 2013. Available online: http://www.nice.org.uk/guidance/cg156

NVOG (Dutch Society of Obstetrics and Gynaecology). Guideline: Male subfertility. *NVOG-richtlijn*, 2010. Available online: http://nvog-documenten.nl/index.php?pagina=/richtlijn/pagina.php&fSelectTG_62=75&fSelectedSub=62&fSelectedParent=75

NVOG (Dutch Society of Obstetrics and Gynaecology). Guideline: Assessment and treatment for male subfertility. *NVOG-richtlijn*, 1999;**17**:1-5. Available online: http://nvog-documenten.nl/uploaded/docs/17_onder_behan_mannesub.pdf

NVOG (Dutch Society of Obstetrics and Gynaecology). Smeenk, JMJ. *IVF-cijfers per centrum 2013*, 2014. Available online: http://www.nvog.nl//Sites/Files/0000004003_IVF_cijfers_2013_per_kliniek.pdf

Otani T, Roche M, Mizuike M, Colls P, Escudero T and Munné S. Preimplantation genetic diagnosis significantly improves the pregnancy outcome of translocation carriers with a history of recurrent miscarriage and unsuccessful pregnancies. *Reprod Biomed Online* 2006;**13**:869-874.

Patrat C, Bienvenu T, Janny L, Faure AK, Fauque P, Aknin-Seifer I, Davy C, Thiounn N, Jouannet P and Lévy R. Clinical data and parenthood of 63 infertile and Y-microdeleted men. *Fertil Steril* 2010;**93**:822-832.

Riccaboni A, Lalatta F, Caliari I, Bonetti S, Somigliana E and Ragni G. Genetic screening in 2,710 infertile candidate couples for assisted reproductive techniques: results of application of Italian guidelines for the appropriate use of genetic tests. *Fertil Steril* 2008;**89**:800-808.

Ruangvutilert P, Delhanty JD, Rodeck CH and Harper JC. Relative efficiency of FISH on metaphase and interphase nuclei from non-mosaic trisomic or triploid fibroblast cultures. *Prenat Diagn* 2000;**20**:159-162.

Rubio C, Rodrigo L, Mir P, Mateu E, Peinado V, Milán M, Al-Asmar N, Campos-Galindo I, Garcia S and Simón C. Use of array comparative genomic hybridization (array-CGH) for embryo assessment: clinical results. *Fertil Steril* 2013;**99**:1044-1048.

Schinzel A. Catalogue of unbalanced chromosome aberrations in man. 2nd edn, 2001. De Gruyter, Berlin, Germany.

Scriven PN and Bossuyt PMM. Diagnostic accuracy: theoretical models for preimplantation genetic testing of a single nucleus using the fluorescence in situ hybridization technique. *Hum Reprod* 2010;**25**:2622-2628.

Scriven P, Flinter F, Khalaf Y, Lashwood A and Mackie Ogilvie C. Benefits and drawbacks of preimplantation genetic diagnosis (PGD) for reciprocal translocations: lessons from a prospective cohort study. *Eur J Hum Genet* 2013;**21**:1035–1041.

Stegen C, van Rumste MM, Mol BW and Koks CA. The value of chromosomal analysis in oligozoospermic men. *Fertil Steril* 2012;**98**:1438-1442.

Stengel-Rutkowski S, Stene J and Gallano P. Risk estimates in balanced parental reciprocal translocations. *Monographie des Annales de Génétique* 1988 ;**14**:1-147.

Tiboni GM, Verna I, Giampietro F, Leonzio E and Impicciatore GG. Cytogenetic findings and reproductive outcome of infertile couples referred to an assisted reproduction program. *Gynecol Endocrinol* 2011;**27**:669-674.

Treff NR, Levy B, Su J, Northrop LE, Tao X and Scott RT,Jr. SNP microarray-based 24 chromosome aneuploidy screening is significantly more consistent than FISH. *Mol Hum Reprod* 2010;**16**:583-589.

van Echten-Arends J, Mastenbroek S, Sikkema Raddatz B, Korevaar J, Heineman M, van der Veen F and Repping S. Chromosomal mosaicism in human preimplantation embryos: a systematic review. *Hum Reprod Update* 2011;**17**:620-627.

Wells D and Delhanty JD. Comprehensive chromosomal analysis of human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization. *Mol Hum Reprod* 2000;**6**:1055-1062.

Wilton L, Thornhill A, Traeger Synodinos J, Sermon KD and Harper JC. The causes of misdiagnosis and adverse outcomes in PGD. *Hum Reprod* 2009;**24**:1221-1228.

Yang Z, Zhang J, Salem SA, Liu X, Kuang Y, Salem RD and Liu J. Selection of competent blastocysts for transfer by combining time-lapse monitoring and array CGH testing for patients undergoing preimplantation genetic screening: a prospective study with sibling oocytes. *BMC Med Genomics* 2014;**7**:38-8794-7-38.

Zhang Y, Zhu S, Wu J, Liu S and Sun X. Quadrivalent asymmetry in reciprocal translocation carriers predicts meiotic segregation patterns in cleavage stage embryos. *Reprod Biomed Online* 2014;**29**:490-498.